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NUTRITIONAL REQUIREMENTS OF MARINE LARVAL AND JUVENILE FISH (FINAL REPORT)

K. L. Simpson, et al

Rhode Island University
Kingston, RI

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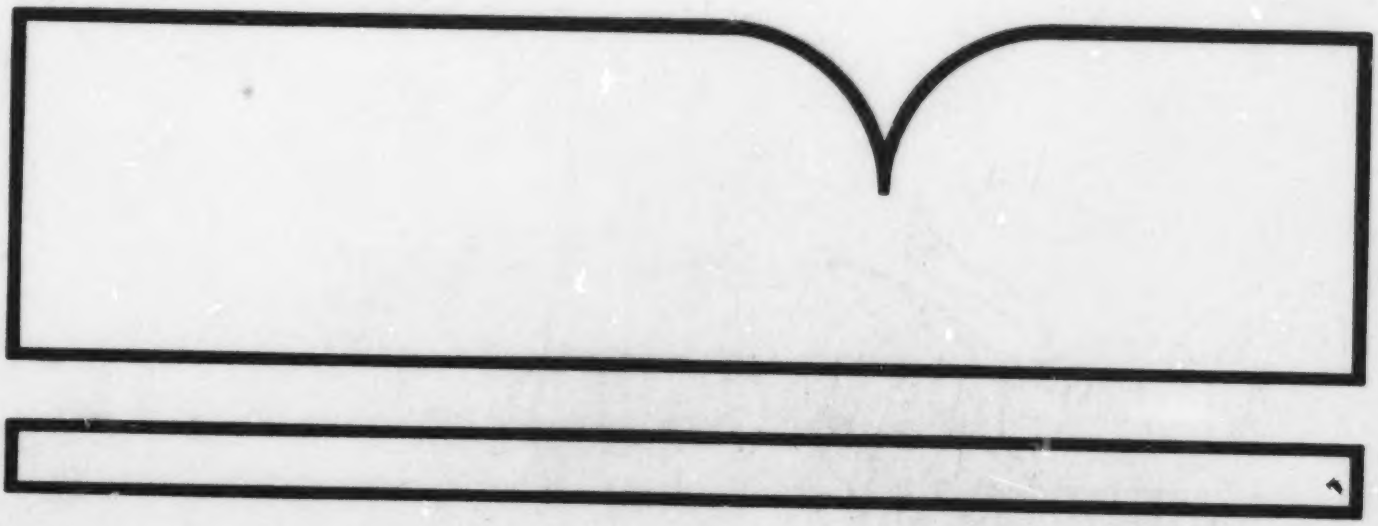

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NUTRITIONAL REQUIREMENTS OF MARINE LARVAL AND JUVENILE FISH

by

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Grant R803818

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OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
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16. ABSTRACT

In 1975, a comparative research effort was initiated to evaluate a variety of diets and diet regimes for the laboratory culture of a marine larval fish. The Atlantic silverside (Menidia menidia) was chosen on the basis of its regional availability, ease of handling in the laboratory and its use as a bioassay organism.

A major consideration was to develop a diet that would provide good growth and survival and provide a test fish similar to its wild counterpart.

Silversides cultured on artificial diets generally had poor growth and survival rates. Freeze-dried brine shrimp (Artemia) nauplii and ground-up adult silversides also failed to promote good growth and survival. On the other hand, live, 3-day-old brine shrimp nauplii provided a diet superior to any artificial ration. However, a combination diet of brine shrimp and artificial diets provided growth and survival more similar to the 'all live' brine shrimp diet even when the live diet was fed only every eighth day. Overall, growth and survival data showed that unstressed fish could be cultured on a combination artificial and brine shrimp diet in as little as a 7:1 day (artificial:brine shrimp) feeding ratio.

Biochemical data were compared to reported requirement levels for essential amino acids and essential fatty acids in marine fish, and it was concluded that all diets seemed adequate in these two components. These data also indicated that fish accepted both artificial and live diets equally. Results further showed that dietary amino acids were generally not stored by silversides beyond levels required for tissue formation, whereas dietary fatty acids were accumulated to a much greater degree. Although the reason for the live diet's nutritive superiority remains unclear, several conclusions can be drawn from these results.

1. Amino acids, fatty acids and digestive enzymes (in the live food) appear not be be limiting in the laboratory rations.

2. A combination live diet and formulated diet provided good growth and survival of unstressed fish having a composition more closely resembling the wild fish.

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FOREWORD

The Environmental Research Laboratory of the U.S. Environmental Protection Agency is located on the shore of Narragansett Bay, Rhode Island. In order to assure the protection of marine resources, the laboratory is charged with providing a scientifically sound basis for Agency decisions on the environmental safety of various uses of marine systems. To a great extent, this requires research on the tolerance of marine organisms and their life stages as well as of ecosystems to many forms of pollution stress. In addition, a knowledge of pollutant transport and fate is needed.

A current objective at ERLN is to develop and verify acute and chronic test methods to determine water quality criteria requirements for protection of indigenous marine life. This requires the capability for culturing the early life stages of marine animals from many phylogenetic groups, specific habitats and trophic levels. The number and diversity of test species must be adequate to support toxicological research providing the data base for developing national marine water quality criteria, site-specific criteria and standards, and marine permit programs such as estuarine and ocean waste disposal.

This study was accomplished as part of an overall effort to determine nutritional requirements for several species of fish suitable for use in toxicological studies. A standard prepared (artificial) diet was formulated and tested for use with larval marine fishes. The suitability of this diet was evaluated against a known successful diet of live brine shrimp, Artemia, nauplii. The results demonstrated that the best available prepared diets can be easily standardized, but does not support survival and growth equivalent to the most commonly used live diet. These findings have shifted the current nutrition research to identifying the diet quality of various available brine shrimp geographical strains. The objective is to characterize and specifiy the most acceptable strain as a reference diet for use in toxicological studies.

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A major consideration was to develop a diet that would provide good growth and survival and provide a test fish similar to its wild counterpart.

Silversides cultured on artificial diets generally had poor growth and survival rates. Freeze-dried brine shrimp (Artemia) nauplii and ground-up adult silversides also failed to promote good growth and survival. On the other hand, live, 3-day-old brine shrimp nauplii provided a diet superior to any artificial ration. However, a combination diet of brine shrimp and artificial diets provided growth and survival more similar to the 'all live' brine shrimp diet even when the live diet was fed only every eighth day. Overall, growth and survival data showed that unstressed fish could be cultured on a combination artificial and brine shrimp diet in as little as a 7:1 day (artificial:brine shrimp) feeding ratio.

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All feeding experiments were carried out at the Environmental Protection Agency, Environmental Research Laboratory at Narragansett, RI (ERLN), by EPA personnel. These studies have been published in the scientific literature and are referenced, but data are also provided here for a more complete presentation of the cooperative research.

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SECTION 1

INTRODUCTION

GENERAL

Interest in the laboratory rearing of marine test fish has increased in recent years. Tarzwell (1969) suggests that laboratory bioassays should be accomplished using the most sensitive life stage of important species, in order to provide tolerance data to set safe levels of a toxicant sufficient for the protection of the organism's whole life cycle. Larval and juvenile stages then are of particular interest for water quality research because of their potentially greater susceptibility to a stressing environment.

Condition of experimental animals is an important factor in water quality research in which the animal's response during exposure to toxicants is studied in laboratory bioassays. Differences in nutritional status of the experimental animal may result in significant variability in tolerances to the experimental stress. Wood et al. (1957) suggest that diet alone is probably the "most important single factor in producing the changes observed in body composition." Development of laboratory-prepared natural or artificial foods would allow precise quality control of diets, thus enabling standardization of nutritional status, subsequent organism condition and response to experimental factors.

Attempts to rear marine fishes have been well documented by May (1971, 1972). A primary problem is a "suitable food supply in conjunction with reasonably good water quality" (Houde, 1972). May (1970) describes a suitable food as one which the larvae will consume and which will contribute to growth. It is also one that is readily available in sufficiently large, quality-controlled amounts. The food must be provided in a succession of continuously increasing sizes, at appropriate concentrations as well as having nutritional adequacy for the growing larvae. A prevalent hypothesis is that size and density of food available to early larval stages may be a factor in the variable abundance of adults (O'Connell and Raymond, 1970; Wyatt, 1972).

The large variety of cultured and prepared foods that have been used in the laboratory to feed fish larvae, and the varying degrees of success, have been summarized by May (1970). This review clearly indicates that a basic knowledge of the nutritional requirements of larval and juvenile marine fish is lacking at present. This poses a serious limitation for future efforts in laboratory rearing of marine fish.

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"Each animal is only what it eats and the source of nutrients to nurture the cells to perform vital functions must come from the food ingested" (Halver, 1968). It is thus possible to have laboratory reared animals that do not biochemically resemble their environmental counterpart. It follows that these fish might also respond quite differently to the stress induced by added toxic chemicals. It is incumbent that the nutritional status should be comparable to that of the same species under 'natural' conditions. A project designed to evaluate artificial diets should provide information on the survival and growth of the test fish on the diet as well as data on the effect of the diet on the biochemical composition of the organism.

As a standard scale to develop and evaluate the nutritional value of feed protein, Phillips and Brockway (reported in Deshimaru and Shigeno, 1972) suggested that the amino acid composition of the diet be patterned after the animal or its egg. Deshimaru and Shigeno support this suggestion in their development of a diet for the marine prawn (Penaeus japonicus). A number of authors have shown that the lipid composition of a marine fish is closely related to its diet. The lipid character of the fish is probably more related to diet than is the protein of fish to protein in the diet.

The growing interest in aquaculture has brought to view the need for nutritionally-sound fish diets that will yield desirable, economical products. In the past, live feeds have been the preferred diet for culturing marine organisms for at least part of the life cycle. Girin has suggested economizing the present use of live brine shrimp (Artemia) as an aquacultural diet by replacing it with an artificial diet by an early 'weaning' of marine fry from brine shrimp.

STUDY SPECIES

Atlantic silversides (Menidia menidia) are euryhaline fish of importance as forage for piscivorous fish including such commercial valuable species as striped bass, bluefish, cod, haddock, mackerel and summer flounder (Bigelow and Schroeder, 1953; Hennekey, R., personal communication, Environmental Research Laboratory, U.S. EPA, S. Ferry Rd., Narragansett, RI 02882). All life history stages of silversides are found in the estuaries along the Atlantic coast. The time of spawning varies with latitude, the peak spawning period in the Chesapeake Bay area being May and the peak in New England being June (Bean, 1903; Bayliff, 1950). The populations of juveniles and adults are observed in decreasing numbers in the late fall. Either mortality, offshore migration or inshore migration is responsible for the near disappearance of estuarine schools. The reported natural diet of silversides includes algae, detritus and zooplankton, such as megalops larvae, copepods and mysids (Hildebrand, 1924; Bayliff, 1950; Carr and Adams, 1973).

STUDY OBJECTIVES

The purpose of the University of Rhode Island's part of the study was to provide biochemical evaluations of the diets and the cultured organisms. The diets were formulated to specifications worked out by URI, EPA and Tunison laboratory personnel. The diets came from feeds formulated by the group as well as those supplied by others.

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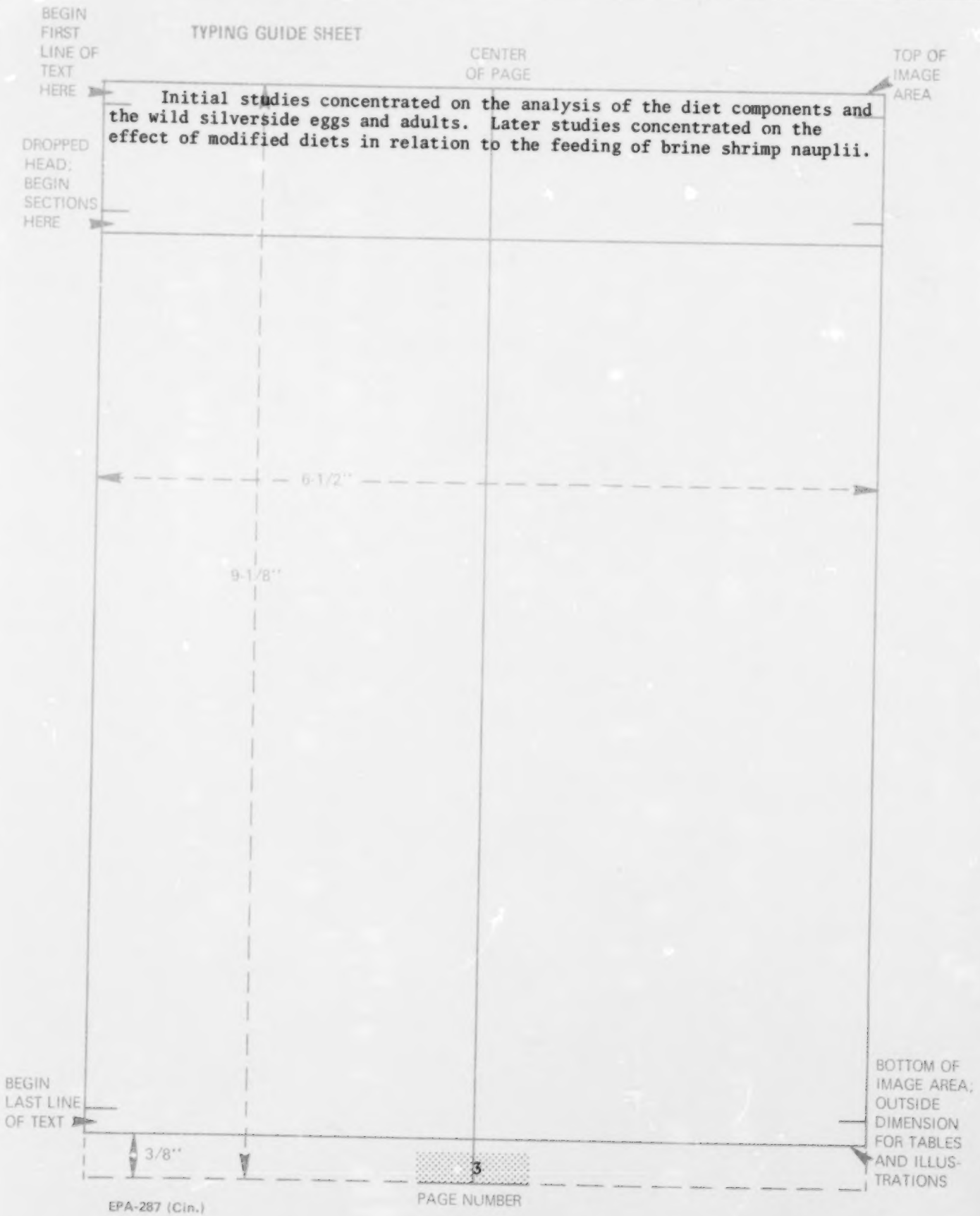
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Initial studies concentrated on the analysis of the diet components and the wild silverside eggs and adults. Later studies concentrated on the effect of modified diets in relation to the feeding of brine shrimp nauplii.
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SECTION 2

METHODS AND MATERIALS

CULTURE

The procedures and systems utilized in the laboratory spawning, fertilization and incubation of silverside eggs have been reported by Barkman and Beck (1976). A detailed description of the fish culture operations has been presented by Beck (in press) and Bengston et al. (1978). The following is a brief synopsis of the above referenced procedures.

Gravid Atlantic silversides were collected from Bissel Cove, Narragansett Bay (Rhode Island) in late May and early June of each year, and transported in aerated containers to holding tanks located at the Environmental Protection Agency Laboratory, Narragansett, Rhode Island. Fish were fed adult brine shrimp during the short holding period. Ripe females were spawned onto nylon monofilament screens with a mesh size of 400 μ and the eggs were subsequently fertilized and suspended in circular, 3-foot diameter hatching jars (Barkman and Beck, 1976).

Wild juvenile silversides were collected with a beach seine from the Pettaquamscutt River, off Narragansett Bay, at various times throughout the summer and fall of each year. These fish were transported, in a similar fashion as the gravid adults, to the laboratory where they were weighed, measured and prepared for analysis.

Culture jars used for the diet study were supplied with a flow-through source of natural filtered seawater at 0.5 liters/min. The temperature of the seawater ranged from 19.7 to 24°C while the salinity fluctuated from 31.4 to 32.2 ppt. The dissolved oxygen levels varied from 5.1 to 6.6 ppm throughout the study period (Bengston et al., 1978). The culture jars were 15 cm in diameter by 46 cm in height and were modified from the original design of Buss (1959), by addition of a bottom center drain. The water circulated through the container in an upflow manner enabling maximum feed suspension.

Test fish were fed 3-day-old brine shrimp previous to the onset of each experiment. They were then cultured on the experimental treatments. Each culture vessel was supplied with 50 fish. Three replicates were run for each treatment. To insure adequate samples for biochemical analyses the total experiment was terminated when 50% mortality was observed in any one experimental culture group. All fish were starved for 24 hours prior to sacrifice to reduce stomach contents. At the conclusion of each experiment the fish were measured, weighed and stored at -20°C under nitrogen until analysis. Each experiment year consisted of two experiments of variable duration.

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DIETS

The diets and treatments used throughout the study consisted of various artificial diets, live 3-day-old brine shrimp, wild plankton, freeze-dried brine shrimp and silversides, and unfed controls. The Tunison Laboratory of Fish Nutrition, U.S.F.W.S., Cortland, NY, formulated several diets. Other diets were obtained from Dr. Samuel P. Meyers, Louisiana State University, Baton Rouge, LA, Dr. Douglas Conklin, Bodega Marine Laboratory, Bodega Bay, CA, and Tetra Marin Staple Food, Tetra Werke Melle, West Germany. Brine shrimp cysts (San Francisco Bay Brand) were obtained from San Francisco Fish Farms, Menlo Park, CA (lot numbers 313/3006 and 321995). The actual diets and their compositions varied from experiment to experiment. The compositions of the various formulated diets are presented in the Results section.

PROTEIN HYDROLYSIS AND AMINO ACID ANALYSIS

A sample from each of the three replicates was acid hydrolyzed in duplicate. The procedure involved the in vacuo hydrolysis of protein material at 110°C for 22 hr with 3.0 ml of 6.0 N HCl. Heavy-walled, vacuum hydrolysis tubes were prepared for use by washing in a chromic acid cleaning solution, then in a mixture of H_2SO_4 and HNO_3 (3:1), rinsed overnight in deionized water and oven-dried. The hydrolysate was filtered through a Buchner funnel (size 15-M) and rotary-evaporated in vacuo at 37°C. To insure removal of HCl, the residue was wet three times with 1 ml of deionized water and dried in the rotary evaporator. The free amino acid residue was then dissolved in 3-5 ml of pH 2.2 sodium citrate buffer, filtered through a 0.45 μ Millipore filter and stored under nitrogen at -20°C until final dilution was made just prior to time of analysis.

Amino acid analyses were performed on a NC-2P Technicon Auto Analyzer equipped with a 25 x 0.5 cm cationic exchange column packed with Chromobead Resin, Type C-3 (ion exchange capacity of 5 meq/mg; 8% cross-linked). The column was operated at 55°C with a flow rate of 0.5 ml/min. A Columbia Scientific Instruments Supergrator-2 Integrator/Calculator was used to compute the absolute amount of each amino acid using color response factors as determined from an authentic standard mixture of amino acids obtained from Pierce Chemical Co., Rockford, IL.

LIPID EXTRACTION AND ANALYSIS

Total lipid extractions and fatty acid analysis were performed as previously reported (Schauer and Simpson, 1978). Briefly, this involved lipid extractions via the Bligh and Dyer technique as modified by Kates with subsequent methylation with 14% boron trifluoride-methanol (w/v).

Fatty acid methyl esters (FAME) were identified with a flame ionization detector in a single column, Varian Aerograph 1200 gas-liquid chromatography unit isothermally operated at 180°C. FAME were separated on a 3% ethylene glycol succinate polyester-Z column and identified and quantified with an electronic integrator (Hewlett Packard 3380A). A confirmation of the identification was made by analyzing the sample a second time on a 15% diethylene glycol succinate column. Authentic standards and literature values for

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published oils (Ackman and Burgher, 1964) were used for identification of FAME. Cod liver oil was used as a secondary standard (Ackman and Burgher, 1965). Results are presented as FAME weight percents of the total lipid.
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SECTION 3

RESULTS

The joint research project between the University of Rhode Island (URI) and the Environmental Protection Agency (EPA) to determine some nutritional requirements of the Atlantic silverside was begun in July 1975 and concluded in January 1979. Experiments were conducted in the summer of each research year and the samples were analyzed during the off-season. The results are reported by the actual experiment year; i.e., 1975, 1976, 1977.

PRE-DIET TESTING ANALYSES

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Much of the first year's work consisted of the amino acid analyses of various life stages of winter flounder (Pseudopleuronectes americanus; Table 1), and a number of natural food organisms known to be consumed during the early life stages of wild marine fish (Table 2). Winter flounder were initially included as a second test organism but were later excluded from the proposed feeding studies due to laboratory and personnel limitations. The basic reason for the initial sample analyses was to elucidate the amino acid pattern of a number of marine organisms so that an artificial diet of suitable quality could be formulated.

Young winter flounder adults (16-18 cm) varied little in amino acid content from the juveniles (4-5 cm) even though the collections were from wild populations collected six months apart. Winter flounder gut contents, from the same populations as the adults (16-18 cm), were similar to the amino acid content of the fish carcass with the following exceptions--the gut contents were low in lysine and arginine while cystine and methionine were not detected.

Although the moisture (80%), ash (3%) and protein contents (15% wet weight-70% dry weight) of the winter flounder were all similar to the lemon sole and cod (Connell and Howgate, 1959) and the Atlantic salmon (Cowey et al., 1962) the amino acid contents were quite different. Generally the winter flounder had a lower level of most all amino acids. Cowey et al. (1970) found that threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine and arginine were the essential amino acids for sole and plaice. These authors further noted that these fish can synthesize the non-essential amino acids for normal growth but stress, such as reproductive activities, may increase the need for essential amino acids and may even make non-essential amino acids limiting.

Plankton collected from Narragansett Bay showed marked variations in amino acid profiles, even when the samples were taken at only one week intervals.

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TABLE 1. AMINO ACID PROFILES OF VARIOUS FRESHWATER AND SALTWATER SPECIES
(Expressed as gram amino acid per 100 grams protein)

Amino Acid	*1A	1B	1C	1D	2	3	4
Aspartic Acid	8.9	11.8	12.8	11.5	17.94	16.12	11.33
Threonine	4.4	3.9	4.7	5.6	7.76	7.21	5.18
Serine	4.7	4.2	5.7	6.5	7.32	6.48	4.62
Glutamic Acid	17.0	18.4	15.9	13.6	28.07	27.90	16.22
Proline	5.7	3.7	4.5	5.5	6.18	5.52	3.66
Glycine	2.9	7.2	9.8	11.3	4.29	4.37	5.06
Alanine	6.8	6.3	7.8	5.3	7.02	5.91	7.06
Valine	6.9	3.6	4.5	6.0	7.76	7.33	6.45
1/2-Cystine	0.8	0.5	0.2	-	1.92	1.95	1.43
Methionine	2.3	3.4	2.8	+	5.80	3.58	3.24
Cystathione	+	+	+	-	-	-	-
Isoleucine	5.7	4.2	3.8	4.2	8.24	7.34	5.71
Leucine	11.1	8.9	8.2	6.1	13.04	13.94	9.37
Tyrosine	3.6	2.7	3.7	3.9	8.49	8.28	4.14
Phenylalanine	4.2	4.1	2.8	4.6	9.25	8.87	4.66
Lysine	7.5	8.4	4.7	5.9	8.93	8.60	10.23
Histidine	0.9	1.1	1.0	3.0	2.13	1.24	2.76
Arginine	6.7	7.6	7.1	6.9	3.38	3.33	6.27

*1) Winter flounder: a) unfertilized eggs, 69% protein
b) wild juveniles, 66.9% protein
c) wild adults, 66.8% protein
d) stomach contents from wild adults.

2) Lemon sole (Connell and Howgate, 1959--recalculated by L.M.R.)

3) Cod (Connell and Howgate, 1959--recalculated by L.M.R.)

4) Atlantic salmon (Cowey et al., 1962--recalculated by L.M.R.)

+) Present, amount not estimated
-) not detected.

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TABLE 2. AMINO ACID PROFILES OF FOOD MATERIALS
(Expressed as gram amino acid per 100 gram protein)

Amino Acid	*1	2	3	4	5
Aspartic Acid	9.9	9.3	11.0	8.0	8.7
Threonine	3.5	4.2	6.5	3.3	4.5
Serine	4.9	4.9	4.4	3.9	3.7
Glutamic Acid	12.5	16.6	12.9	14.4	13.9
Proline	4.8	-	6.2	5.0	3.1
Glycine	6.1	5.7	4.9	6.2	5.4
Alanine	6.6	8.0	7.0	6.7	11.8
Valine	6.5	6.3	5.7	5.5	5.5
1/2-Cystine	.4	-	.3	+	.6
Methionine	2.9	7.1	2.3	2.2	2.5
Cystathione	+	-	+	+	-
Isoleucine	4.4	5.2	4.8		1.7
Leucine	10.5	8.2	6.8	10.2	6.4
Tyrosine	3.7	5.9	4.2	4.7	6.8
Phenylalanine	6.4	4.6	5.5	7.5	5.0
Lysine	7.3	4.9	7.9	9.9	8.3
Histidine	1.3	1.4	2.0	.6	2.0
Arginine	8.1	7.5	7.3	11.9	10.1

*1) Skeletonema costatum - cultured by Dr. Smayda of U.R.I.: 54.8% protein

2) Plankton-estuary - collection 4/1[illegible]; 61.2% protein

3) Plankton estuary - collection 4/[illegible]1; copepod larvae, barnacle nauplii, rotifers

4) Plankton-bay - collection 4/9; 51.1% protein

5) Plankton-bay - collection 4/16; 68.9% protein; crustaceans

+) Present, amount not estimated
-) Not detected.

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Alanine, tyrosine and histidine showed the widest variations. A similar relationship was seen between sample collections from the Narrow River, although it is important to consider that these collections were from different sample locations. The levels of amino acids in the Skeletonema sample generally fell within the range of values found in the plankton samples.

We assumed that the levels of amino acids in the winter flounder eggs were optimum for growth. Based upon the resemblance of the plankton amino acid profile to that of the winter flounder egg, the plankton appeared to be an excellent diet. Plankton was therefore included as a diet in study year 1976.

FEEDING STUDY 1975

The initial study year was comprised of two separate investigations. One study was undertaken to analyze the effect of a continuous live brine shrimp diet on larval 60+ day-old juveniles. The second study was initiated to evaluate a practical, commercial hatchery diet, Cortland #1 (Agway, Inc.), which was formulated with respect to the nutritional requirements of a freshwater fish, the Atlantic salmon fry. This diet could be used as a basic starting point toward the development of a nutritionally adequate, artificial marine fish diet. Brine shrimp nauplii which are known to sustain laboratory reared larval silversides in the past were used as a reference (standard) diet.

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Live Brine Shrimp Feeding

After hatching, the fish were transferred to a 720 liter holding tank and fed live 3-day-old brine shrimp. Fish were periodically removed during the two month batch culture study for biochemical analyses.

The total protein levels and amino acid profiles are given in Table 3 for the wild silversides, their eggs, and laboratory cultured fish of various ages. The amino acid spectrum of the silversides was very similar to the spectrum of migrating Atlantic salmon (Cowey et al., 1962).

The brine shrimp (diet) analysis was similar to the results of Gallagher and Brown (1975) who also analyzed San Francisco Bay brine shrimp. These authors stated that methionine in the brine shrimp may be limiting compared to standard egg albumin levels. However, our results showed that the methionine levels in brine shrimp were very similar to the level found in the silverside eggs. The major differences between the 3-day-old brine shrimp and the silverside eggs were the lower levels of threonine, serine, proline, valine, and leucine, and the higher levels of arginine in the brine shrimp.

The silverside eggs contained higher levels of threonine, serine, proline, alanine, leucine, and tyrosine, and lower levels of glycine and methionine than were found in the wild fish. The amino acid profile of the 5-day-old silversides changed substantially from the profile of the eggs. Most of the changes resulted in a general decrease in amino acids from the egg to the larval stage.

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TABLE 3. AMINO ACID PROFILES OF SILVERSIDES
(Expressed as gram amino acid per 100 gram protein)*

Amino Acid	Wild Silversides	Eggs Unfert.	Cultured Silversides 5-day	10-day	25-day	58-day	Brine Shrimp Diet
Aspartic Acid	9.3	8.7	10.2	9.9	9.9	10.2	8.9
Threonine	4.3	6.4	4.6	5.1	4.7	4.4	3.1
Serine	4.3	8.2	4.9	4.9	5.2	4.6	4.4
Glutamic Acid	14.1	13.7	15.2	14.4	15.2	13.8	11.6
Proline	3.9	7.5	4.2	4.3	4.5	4.8	3.1
Glycine	6.0	3.7	5.8	6.0	6.4	6.6	4.3
Alanine	6.0	7.7	4.7	4.5	4.9	6.2	6.0
Valine	5.3	6.8	5.5	5.6	5.8	5.6	5.1
Methionine	3.6	2.1	2.5	2.8	2.2	3.3	2.3
Isoleucine	4.6	5.9	4.8	5.1	4.4	4.0	4.9
Leucine	7.7	10.6	7.7	7.8	7.2	7.5	8.9
Tyrosine	3.5	5.1	3.9	4.0	3.9	3.2	4.2
Phenylalanine	4.2	4.7	4.7	4.6	4.6	4.3	4.5
Histidine	3.6	2.9	2.9	2.9	2.7	5.1	1.2
Lysine	8.9	8.3	8.3	8.7	8.3	8.9	9.2
Arginine	6.3	7.1	7.3	7.1	7.3	6.5	10.5
% Protein†	15	NA§	13	12	NA	14	NA
% Moisture	NA	84	79	81	81	79	NA

* Data from Simpson et al., 1979.

† Wet weight basis

§ NA = not available

Tryptophan was not determined by the procedure used in this study.

From the 5th to the 58th day of culture, the amino acid profiles did not change markedly. The only changes which occurred were a decrease in glutamic acid and an increase in alanine and histidine. Compared to the 3-day-old brine shrimp diet, the 58-day-old fish differed only in the levels of histidine and arginine. Therefore, it seems that the dietary amino acids were absorbed and deposited as early as the fifth day of life and did not change drastically thereafter.

The percent composition of the fatty acids, the percent total lipid and the total ω3/ω6 ratios of the Atlantic silverside eggs, laboratory cultured fish, the wild silverside and the live 3-day-old brine shrimp nauplii diet fed for 137 days are presented in Table 4. Included with the various age groups of the fish are their corresponding sizes in length and weight.

Unfertilized eggs have a whole body lipid level of 13.9%. The two acids, 20:5ω3 and 22:6ω3, comprised more than 35% of the total fatty acids and the ω3 acids the ω6 component by greater than 7.5 times. It would appear that the high energy level and the large ω3 component are both indicative of their metabolic and physiological importance in the early life stages.

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TABLE 4. PERCENT COMPOSITION OF FATTY ACIDS* OF SILVERSIDES CULTURED ON LIVE, THREE-DAY-OLD BRINE SHRIMP NAUPLII (Lengths, mm, and weights, mg, are in parentheses)§

Fatty Acid	Eggs	2-day (6.0mm) (Sac-fry)	15-day (11.0mm) ------	23-day (13.1mm) (8.90mg)	58-day (22.2mm) (22.2mg)	72-day (26.1mm) (77.6mg)	109-day (34.6mm) (209.0mg)	137-day (43.3mm) (387.0mg)	Brine Shrimp Diet	Wild (43.6mm) (346.0mg)
14:0	2.50	1.14	0.69	0.53	1.04	0.69	1.24	1.64	1.16	1.37
14:1	0.27	0.06	0.30	0.29	0.50	0.35	0.59	0.76	1.10	0.19
15:0	0.77	0.36	0.33	0.32	0.46	0.41	0.64	0.60	0.53	0.84
15:1	0.15	0.02	0.19	0.09	0.39	0.27	0.29	0.34	0.38	0.30
16:0	18.67	22.67	16.29	16.77	20.29	17.44	17.84	14.99	11.45	19.47
16:1	7.40	4.48	10.06	8.35	11.43	8.46	12.59	15.96	16.49	4.83
17:1	1.28	0.13	1.49	0.90	1.43	0.87	-	2.87	2.72	0.43
18:0	5.99	9.74	7.34	8.72	6.14	8.22	8.53	9.01	4.10	8.50
18:1ω9	14.19	12.83	25.33	27.44	38.46	27.94	33.72	36.34	34.34	11.69
18:2ω6	1.58	0.68	2.24	2.36	3.15	2.60	2.52	2.24	4.78	1.36
18:3ω6	0.55	0.32	0.52	0.42	0.94	0.94	0.83	0.60	0.75	0.53
18:3ω3	1.47	0.44	2.49	2.85	2.29	3.17	2.34	2.51	4.67	0.86
18:4ω3	0.88	0.47	0.34	0.26	-	0.35	0.49	0.68	0.73	1.12
20:1ω9	1.36	0.20	0.49	0.33	1.03	0.68	1.01	0.85	0.55	1.18
20:4ω6	2.71	2.52	5.13	5.99	3.79	5.89	3.60	1.98	3.13	3.78
20:5ω3	8.09	4.90	7.84	7.89	2.93	6.13	4.70	4.21	13.31	8.93
22:5ω6	0.49	-	-	-	-	-	-	-	-	1.19
22:5ω3	3.72	2.33	3.26	2.23	0.92	4.22	2.70	1.59	-	2.12
22:6ω3	27.15	36.05	14.96	13.67	4.42	11.06	5.84	1.95	-	29.93
% oil†	13.92	-	-	12.88	12.37	10.84	14.71	21.45	-	8.45
ω3/ω6 ratio	7.52	12.55	3.66	3.07	1.34	2.62	2.31	2.23	2.16	5.93

* Weight percent values.

† Based on dry weight.

§ Data from Schauer, 1977.

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In the 2-day-old yolk-sac fry, the 16:0, 18:0, and 22:6ω3 were the major acids which were preferentially selected from the energy rich egg, while 16:1, 18:1ω9, and 20:5ω3 were the major fatty acids which showed reduced levels. A similar pattern of fatty acid retention and utilization was found by Hayes (1972) and his associates in the total lipids of the developing steelhead (Salmo gairdneri). The resulting increase in 22:6ω3 caused a rise of the 7:52ω3/ω6 ratio of the egg to 12:55 in the fry.

The brine shrimp diet was composed largely of 16:0, 16:1, 18:1ω9, and 20:5ω3 and contained no 22:6ω3. This analysis relates well to reports by Kayama et al. (1963), Enzler et al. (1974), and Gallagher and Brown (1975). However, they differ from those of Weaver (1974) and Benijts et al. (1975) who found higher levels of 18:3ω3 and little or no 20:5ω3. The reason for these differences may be due to the dietary components of the parental brine shrimp, and further points out the reason for a standard diet.

The analyses of silversides cultured on the live brine shrimp nauplii diet showed that the fatty acids, 16:1 and 18:1ω9, increased substantially from the 2-day-old yolk-sac fry levels, while 16:0 and 22:6ω3 decreased markedly. Since the diet contained a large amount of 16:1 and 18:1ω9 but no 22:6ω3, it is apparent that the fish modified their concentration of fatty acids, perhaps through direct assimilation, to reflect the composition of the diet. A few notable exceptions were the maintenance of the level of 18:0 even though the dietary level was low, and the lack of accumulation of 20:5ω3, a major fatty acid in the brine shrimp diet. With respect to the latter acid, it is tempting to suggest that it and 18:3ω3 were perhaps utilized as a precursor in the production of 22:6ω3.

The 72-day-old fish have a much higher level of 22:6ω3 than the other groups; however the percent oil at this point is about 2-4% lower than in the other fish groups (58 or 109-day-old fish). The increased 22:6ω3 level may represent a mechanism by which the fish can metabolically concentrate important fatty acids when their lipid levels are lowered, or that perhaps this acid is not utilized as are 16:0, 16:1 and 18:1ω9.

The total ω3/ω6 value of the live brine shrimp nauplii fed fish stabilized at approximately 2.4 in the latter three test points. This may be the base line level at which biosynthetic conversions become important as a means of maintaining their tissue levels of 22:6ω3, provided their dietary levels of 18:3ω3 and 20:5ω3 are sufficient to supply the needed precursors. The brine shrimp diet would seem to satisfy that requisite.

Compared to the wild fish, the cultured fish have a far lower ω3 and a much higher ω6 fatty acid level. The ω3/ω6 ratio of the wild fish lipid averaged 6.8, over three times the cultured fish levels. The wild fish fatty acid profile was similar to the egg and 2-day-old yolk-sac fry values. The fatty acids 20:5ω3 and 22:6ω3 represented about 40% of the total fatty acid composition in the wild fish.

As the ω3 PUFA levels decreased in the brine shrimp fed group, the total lipid level increased. A level of 21.4% total lipid was established by the

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end of the experiment. Contrary to the high levels in cultured fish, the wild fish lipid level was about 8.5% of the total body weight.

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Artificial Diet Study

Juvenile silversides (28 days old - 15 mm) previously reared on brine shrimp nauplii, rotifers and phytoplankton were separated into two groups of 80 fish each. One group was fed the powdered Cortland #1 diet and a second group was fed 3-day-old brine shrimp nauplii. At an age of 75 days (47 days of feeding) the record showed the following:

	Cortland #1	Brine shrimp nauplii
Cumulative mortality	38%	5.0%
Growth	11.4 mm (15.0-26.4)	18.2 mm (15.0-33.2)

Approximately 25% of the Cortland #1 fed fish exhibited signs of scoliosis and/or lordosis and mortality increased rapidly after approximately 22 feeding days. At the 75 day point, some of the Cortland fed fish were switched to the brine shrimp diet to determine if this diet would stabilize the apparent nutritional problem. Shortly after the transition was made, mortalities leveled off and the growth rate began to increase. The vertebral problems were, however, not corrected.

Two factors which could have contributed to the high mortality and presence of vertebral deformities may have been the leaching of dietary components from the artificial diet when it became water soaked, or perhaps an inadequate lipid composition (soybean oil). In reference to the latter point, the fatty acid composition of the artificial diet is shown in Table 5 along with the spectrum for soybean oil and that of silversides cultured on the salmon type diet. The soybean oil diet and the oil resemble each other to some degree, since 16:0, 18:1ω9, and 18:2ω6 are the major fatty acids of both analyses. Likewise, silversides fed this artificial diet closely resemble the lipid make-up of the diet they were fed. However, it is evident that fish fed the artificial diet were not similar in fatty acid composition to the wild fish, and thus this diet had not accomplished the major goal of providing a laboratory cultured fish of similar biochemical composition to the wild fish (Table 4).

The soybean oil resulted in a ω3/ω6 ratio in silversides of only 0.36 which is far below the level found in wild silversides, or even brine shrimp-fed silversides after 137 days (Table 4). The high level of 18:2ω6 and low level of ω3 fatty acids in the fish could possibly be modified if the artificial diet was more closely like that of the wild silversides. With this in mind, a number of marine oils were evaluated for their suitability as a lipid source in future diet formulations. The fatty acid profiles of three marine oils are presented in Table 6 and show that, in all cases, marine oils are higher in the long chain polyunsaturated fatty acids (PUFA) commonly found in wild fish. The difficulty with supply of the red crab and the high level of 22:1ω9 in the herring oil caused their exclusion from future diets.

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Therefore, cod liver oil was selected as the best artificial diet lipid source.

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TABLE 5. THE MAJOR FATTY ACIDS OF SOYBEAN OIL, THE ARTIFICIAL DIET WITH A SOYBEAN BASE, AND SILVERSIDES CULTURED ON THE DIET*

FAME	Artificial Diet With Soybean Oil Cortland #1	Soybean Oil	Silversides Fed the Artificial Diet (13.97 mm long)
16:0	13.05	12.52	17.43
16:1	2.43	-	3.74
18:0	2.45	4.69	7.41
18:1ω9	36.12	19.25	36.37
18:2ω6	29.81	54.90	21.22
18:3ω3	1.88	7.83	0.51
20:1ω9	2.94	-	2.95
20:5ω3	1.62	-	-
22:6ω3	2.08	-	4.76
ω3/ω6 ratio	0.21	0.20	0.36

* Data from Simpson et al., 1979.

TABLE 6. FATTY ACID CONSTITUENTS OF VARIOUS OILS

Fatty acid Methyl ester	1	2	3
14:0	2.45	8.75	5.45
15:0	1.98	0.58	0.46
16:0	15.19	15.82	10.81
16:1	6.88	6.77	12.35
16:2/17:1	1.23	0.70	0.70
18:0	5.40	1.26	2.47
18:1ω9	21.66	14.33	27.11
18:2ω6	2.94	1.84	2.02
18:3ω3/20:1ω9	3.59	14.84(20:1ω9)	12.47(20:1ω9)
18:4ω3	-	1.56	1.62
20:4ω3	trace	0.80	0.59
20:5ω3	13.88	4.26	7.58
22:1ω9	6.88	21.11	6.40
22:5ω3	2.06	0.88	0.91
22:6ω3	9.30	4.32	7.09
ω3/ω6 ratio	9.81	6.42	8.81

1) Red Crab (waste) oil - shell and viscera - East Coast product

2) Herring oil with antioxidant - Commercial quality

3) Cod liver oil - medicinal quality

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FEEDING TRIAL 1976

The second year of the project centered mainly on the chemical and biochemical aspects of the diets fed and the analysis of the fish cultured on these diets. The diets consisted of wild plankton, brine shrimp nauplii (fresh and freeze-dried), freeze-dried silversides, Tetra Marin, and four other artificial diets. The latter four diets incorporated changes suggested by the first year's trials, e.g. use of a marine oil and the incorporation of a semi-purified diet with and without an amino acid supplement. Table 7 lists the experimental treatments for the two experiments of this study year. The treatments in experiments 1 and 2 were nearly identical except for the differences noted in Table 7.

TABLE 7. DESCRIPTION OF THE EXPERIMENTAL TREATMENTS EVALUATED IN 1976 SILVERSIDE FEEDING STUDIES
(Each diet was used in both experiments 1 & 2 except where indicated)

Experimental treatments	Source and description*
1. Wild plankton (Experiment #1 only)	Collected in West Passage, Narragansett Bay by conical net with 243 μ mesh opening and retained on a 116 μ mesh sieve.
2. Brine shrimp nauplii, live	San Francisco Bay brine shrimp, Artemia (lot nos. 313/3006 & 321995), eggs hatched and harvested after 72 hrs in filtered and autoclaved seawater of 20-22°C and 20-30 o/oo salinity. Size approx. 140 μ m x 400 μ m.
3. Starved	Unfed
4a. Brine shrimp nauplii, freeze-dried (Experiment #1, only)	Nauplii as obtained in diet #2, then freeze-dried 24 hrs to constant weight.
4b. Silversides adults, freeze-dried (Experiment #2, only)	Menidia menidia were collected from Bissel Cove, Narragansett Bay with a beach siene. They were then freeze-dried for 24 hrs to constant weight.
5. Tetra Marin	Lot #125244. Tetra Marin Staple Food, Tetra Werke Dr. rer. nat. Baensch, Melle, West Germany.†
6. Artificial, CM-1	Cortland #1 diet with cod liver oil. (See Table 8).
7. Artificial, C-1	Cortland #1 diet with soybean oil. (See Table 8).
8. Artificial, CMP-1	Semi-purified diet with cod liver oil. (See Table 9).
9. Artificial, CMP-2	Semi-purified diet with cod liver oil. (See Table 9).

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* Diets #2, 4a, 4b, and 5-9 were ground to a coarse powder which would pass through a 400 μ mesh sieve.

† Unknown proportions of fish meal, crab meal, brine shrimp, mussel meal, lobster meal, halibut meal, beef heart meal, halibut liver, Calanus finmarchicus, kelp, oat flour, wheat germ oil, Agar-Agar, seaweed and bone charcoal.

TABLE 8. COMPOSITION OF THE ARTIFICIAL DIETS, CM-1 AND C-1

Components	Percent Composition*	
	CM-1	C-1
Herring meal	40.00	40.00
Soybean meal	10.00	10.00
Corn gluten meal, 60%	10.00	10.00
Wheat middlings, standard	9.00	9.00
Brewers dried yeast	5.00	5.00
Dried condensed fish solubles	5.00	5.00
Dried whey	5.00	5.00
Meat and bone meal	5.00	5.00
Soybean oil	-----	10.00
Cod liver oil	10.00	-----
Mineral mixture†	0.40	0.40
Vitamin mixture§	0.70	0.70

* Diets were compounded by the Tunison Laboratory of Fish Nutrition, U.S. Fish and Wildlife Service, Cortland, New York.

† Mixture provided the following compounds in g/kg diet: $MgSO_4$, 2.0; $ZnSO_4$-H_2O, 0.3; $FeSO_4$-$7H_2O$, 0.3; $CuSO_4$, 0.3; KIO_3, 0.0091 and $MnSO_4$-H_2O, 1.0.

§ Mixture provided 10,000 IU Vitamin A as retinyl palmitrate; 4,000 IU Vitamin D as Cholecalciferol; 75 IU Vitamin E as dl-α-tocopheryl acetate; and the following amounts (milligrams) of other vitamins per kilogram of diet: menadione dimethylpyriminidol bisulfate, 10.0; thiamine HCL, 4.0; riboflavin, 30.0; calcium pantothenate, 150.0; niacinamide, 300.0; pyridoxine-HCL, 20.0; d-biotin, 6.0; folacin, 15.0; Viatmin B_{12}, 0.002; L-ascorbic acid, 1000; inositol, 500.0; butylated hydroxytoluene (100%), 100.0; and choline chloride (70%), 1330.0.

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TABLE 9. COMPOSITION OF THE ARTIFICIAL SEMI-PURIFIED DIETS, CMP-1 and CMP-2*

Composition	Percent Composition	
	CMP-1	CMP-2
Caesin	40.00	40.00
Gelatin	10.00	10.00
Cod liver oil	10.00	10.00
Sucrose	10.00	10.00
Dextrin, white technical	10.00	10.00
Cellulose†	4.26	7.16
Choline chloride 70%	0.30	0.30
L-glutamic HCL	1.20	-----
L-ascorbic acid	0.30	0.30
L-glutamic acid	6.30	-----
NaCl	0.50	0.50
Mineral mixture§	6.40	6.40
Vitamin mixture††	0.50	0.50
DL-methionine	0.20	0.20
L-tryptophan	0.04	0.04
Amino acid mixture§§	-----	4.60

* Diets formulated at the Tunison Laboratory of Fish Nutrition, U.S. Fish and Wildlife Service, Cortland, New York.

† Solka floc, Brown Company, Berlin, N.H.

§ Minerals in g/kg diet: $CaHPO_4H_2O$, 18.04; $CaCO_3$, 19.04; KH_2PO_4, 14.03; $NaHCO_3$, 8.82; $MnSO_4 \cdot H_2O$, 0.35; $FeSO_4 \cdot H_2O$, 0.50; $MgSO_4$, 3.02; KIO_3, 0.01; $CuSO_4 \cdot H_2O$, 0.03; $ZnCO_3$, 0.15; $CsCl_2 \cdot 6H_2O$, 0.002; $NaMoO_4 \cdot H_2O$, 0.008; and Na_2SeO_3, 0.002.

††The vitamin mixture included 10,000 IU Vitamin A as retinyl palmitate; 4,000 IU Vitamin D as Cholecalciferol; 75 IU Vitamin E as dl-α-tocopheryl acetate; and the following amounts of vitamins in mg/kg diet: thiamin·HCL, 40.0; menadione dimethylpyrimidinol bisulfite (Vitamin K), 2.0; riboflavin, 30.0; D-calcium pantothenate, 150.0; niacin, 300.0; pyridoxine·HCL, 20.0; d-biotin, 0.5; folic acid, 15.0; Vitamin B_{12}, 0.3; Ethoxyquin (100%), 200.0; and myo-inositol, 500.0.

§§Amino acids in g/kg diet: L-threonine, 7.0; L-valine, 5.0; L-cystine, 3.0; L-isoleucine, 10.0; L-leucine, 8.0; lysine·HCL, 2.0; and L-arginine·HCL, 11.0.

Proteins

Table 10 shows the amino acid profile of silversides fed the various test diets. There was generally little amino acid variation in the fish fed any of the seven diets. In the case of the CMP-2 diet fed fish, the values are close to CMP-1 fed fish which received only methionine and tryptophan supplementation. However, it is evident that methionine from the CMP-2 diet was incorporated to a slightly greater degree than from the CMP-1 diet. Nevertheless, growth and survival were unchanged, indicating that other factors must be involved other than the methionine level of the diet. For

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example, supplemental amino acids of the CMP-2 diet may aid the availability of methionine to the fish.

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TABLE 10. AMINO ACID PROFILES OF SILVERSIDES FED THE VARIOUS ARTIFICIAL AND NATURAL DIETS
(Expressed as gram amino acid per 100 gram protein)

Amino Acid	Experimental Diets 1	2	4	5	6	8	9
Aspartic Acid	10.1	9.9	10.5	10.2	10.2	9.7	10.0
Threonine	4.5	4.0	4.9	4.4	4.4	5.0	4.3
Serine	4.5	4.2	5.0	4.7	4.7	5.0	5.4
Glutamic Acid	15.4	16.3	14.8	15.4	14.3	15.2	14.1
Proline	3.9	3.7	4.0	4.6	4.6	4.5	4.7
Glycine	6.7	6.8	7.0	6.6	7.2	6.0	6.4
Alanine	6.4	6.7	6.4	7.4	5.2	5.6	6.2
Valine	5.2	4.5	5.0	5.1	5.4	5.6	5.7
Methionine	1.5	2.4	1.5	2.5	1.9	2.3	3.2
Isoleucine	5.1	4.6	4.8	4.8	4.5	4.2	4.5
Leucine	7.9	11.4	7.8	7.4	7.7	8.1	7.8
Tyrosine	2.3	3.2	2.4	3.3	3.7	3.1	3.5
Phenylalanine	4.5	4.2	4.6	4.4	4.8	4.8	4.9
Histidine	3.3	2.7	3.5	3.4	4.7	3.8	3.3
Lysine	8.5	9.2	9.1	9.0	8.6	7.9	8.1
Arginine	7.3	6.2	7.4	7.3	6.9	7.0	7.1
Ammonia	19.6	16.2	17.8	16.1	17.4	18.4	16.9
% Protein*	19	17	17	16	13	15	14
% Moisture	76	75	NA†	77	NA	77	NA

* Net weight basis

† NA = not available

Another example of differential availability may be seen when comparing brine shrimp with brine shrimp-fed fish (Table 10). The methionine and histidine values are low in brine shrimp nauplii as compared to the fish indicating that these amino acids may be preferentially assimilated from the brine shrimp diet versus the artificial diets.

Lipids

The fatty acid composition of the various experimental diets used are presented in Table 11 along with the $\omega 3/\omega 6$ ratios for each diet. The fatty acid spectrum, percent survival, and weight gains, for experiment 1 test fish are presented in Table 12. Similar data on fish cultured in experiment 2 are shown in Table 13.

Since all treatment 3 (unfed) fish died within 12 days, in experiment 1, and only 32% survival was evident at termination (9 days) in experiment 2, it

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was concluded that the system was providing no nutritional supplement. Treatment 3 in experiment 2 was terminated after only 9 days to ensure adequate samples for biochemical evaluations.

TABLE 11. PERCENTAGE COMPOSITION OF FATTY ACIDS OF THE VARIOUS EXPERIMENTAL DIETS*

Fatty acid	Experimental diets* 1	2	4a	4b	5	6	7	8	9
14:0	7.42	1.16	1.33	2.86	1.39	4.50	1.90	4.39	4.72
14:1	1.64	1.10	1.14	0.30	0.14	0.40	0.16	0.51	0.46
15:0	0.87	0.53	0.57	1.53	0.18	0.31	0.15	0.33	0.30
15:1	0.15	0.38	0.36	0.35	0.06	0.09	0.02	0.13	0.12
16:0	26.30	11.45	12.27	20.12	16.83	13.17	13.00	10.84	11.59
16:1	10.50	16.49	17.05	6.16	4.74	9.03	2.45	11.21	11.48
16:4	0.45	-	-	-	0.06	-	0.08	0.40	0.19
17:1	0.24	2.72	2.55	-	-	0.18	0.06	0.27	0.20
18:0	5.24	4.10	2.16	7.79	3.64	0.66	2.45	2.01	2.02
18:1ω9	9.48	34.46	36.43	11.11	22.40	24.36	35.56	25.05	26.73
18:2ω6	1.93	4.78	2.98	2.25	31.38	7.64	29.52	3.66	2.83
18:3ω6	1.13	0.75	0.81	-	-	-	-	-	-
18:3ω3	2.70	4.67	4.49	1.00	5.31	1.05	1.90	1.04	0.81
18:4ω3	3.46	0.73	0.75	1.62	0.25	1.99	0.70	3.89	2.12
20:0	0.13	-	0.03	0.34	0.18	-	-	-	-
20:1ω9	0.36	0.55	0.62	1.00	3.43	9.40	3.49	9.77	10.28
20:2ω6	0.40	-	0.04	-	0.08	0.19	-	0.27	0.29
20:4ω6	1.22	3.13	2.72	2.94	0.36	0.33	0.11	0.26	0.30
20:5ω3	12.58	13.31	12.20	8.26	3.46	8.36	1.48	9.94	9.45
22:1	-	-	-	-	1.99	6.95	4.41	5.03	5.01
22:5ω3	0.03	-	-	3.46	0.21	0.62	0.06	0.81	0.81
22:6ω3	13.44	-	-	26.21	3.72	9.69	1.99	10.60	10.09
ω3/ω6 ratio	6.88	2.16	2.66	7.11	0.41	2.65	0.21	6.27	6.81

* Tables 11, 12 and 13 data from Schauer, 1977.

In both experiments dietary fatty acids were generally absorbed or converted in such a manner as to resemble the ω3/ω6 values of the diets. Nevertheless, particular acid concentrations did change. The fatty acids 14:0, 16:1, 18:2ω6, 18:3ω3 and 20:5ω3 were generally lower in the fish than the diet, while 16:0, 18:0, 18:1ω9, 20:5ω3 and 22:6ω3 were preferentially retained or synthesized. The metabolic implications of these results are that the acids which were not concentrated were probably biosynthetically interconverted or utilized for energy, while the acids which are preferentially retained or synthesized are specifically required for tissue and cellular membrane development.

Although growth was variable in each treatment some biosynthetic interconversions of 18:3ω3 and 20:5ω3 to 22:6ω3 or a preferential accumulation was evident in most diet fed fish. The cumulative dietary level of 18:3ω3 and

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20:5ω3 was 8.77% while 22:6ω3 was present at 3.72%. In the cultured fish (treatment 5) the distribution was 3.24%, 18:3ω3 and 20:5ω3 and 16.62%, 22:6ω3.

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TABLE 12. PERCENT COMPOSITION OF THE FATTY ACIDS OF SILVERSIDES CULTURED FOR 23 DAYS ON VARIOUS DIETS (EXPERIMENT #1)

Fatty acid	24-day old	Experimental diets 1	2	4a	5	6	7	8	9	Wild Fish (72.90 mg)
14:0	0.53	1.26	1.39	0.90	1.08	2.27	1.46	2.53	3.51	1.34
14:1	0.29	-	0.32	0.44	-	-	0.06	0.17	-	0.14
15:0	0.32	0.56	0.51	0.66	0.52	0.47	0.35	0.45	0.83	0.88
15:1	0.09	0.18	0.14	0.31	0.33	0.15	0.18	0.27	0.48	0.14
16:0	16.77	21.43	15.66	16.91	19.28	17.94	17.95	19.56	20.68	22.55
16:1	8.35	4.13	13.09	8.51	3.14	5.23	2.92	6.36	4.64	5.83
17:1	0.90	-	0.63	1.33	1.88	0.09	0.11	0.99	-	0.14
18:0	8.72	13.31	6.32	6.09	13.58	7.40	7.99	9.16	9.53	9.44
18:1ω9	27.44	12.52	33.83	27.22	15.41	24.27	34.20	24.72	24.60	10.40
18:2ω6	2.36	1.30	3.45	2.85	13.54	3.06	18.39	2.78	1.82	1.23
18:3ω6	0.42	1.95	1.14	1.45	1.71	1.31	2.45	0.58	0.58	0.38
18:3ω3	2.95	0.56	3.05	1.92	1.48	0.48	0.52	0.18	0.80	0.88
18:4ω3	0.26	0.30	0.44	0.76	0.46	0.42	0.21	0.36	0.36	0.75
20:0	0.20	0.38	0.03	0.21	1.34	-	-	0.18	0.15	-
20:1ω9	0.33	1.14	0.79	1.35	1.74	7.81	2.78	6.10	5.58	0.71
20:2ω6	-	0.46	-	0.06	0.29	0.68	0.08	0.05	0.15	0.09
20:4ω6	5.99	3.32	3.93	5.37	3.49	2.66	1.48	2.97	2.54	3.75
20:5ω3	7.89	5.14	4.60	6.89	1.76	3.00	0.44	2.42	2.22	7.37
22:1	-	0.14	-	0.37	0.51	3.91	1.27	2.28	2.44	0.20
22:5ω3	2.23	2.60	4.70	2.63	1.30	0.55	-	0.65	1.47	1.34
22:6ω3	13.67	29.03	5.83	13.57	16.62	17.47	6.95	17.24	17.99	35.53
% oil	12.88	11.27	19.90	10.97	11.72	12.82	-	15.45	12.44	-
ω3/ω6 ratio	3.07	5.15	2.18	2.65	1.12	2.84	0.36	3.27	4.46	7.53
% survival*	-	54.00	97.00	66.00	95.00	51.00	28.00	65.00	61.00	-
weight gain*	-	4.30	36.60	4.50	4.40	0.60	1.20	-0.70	-0.50	-

* Data from Beck and Poston (in press). Weight increase in mg over 8.9 mg initial weight.

Fish fed live 3-day-old brine shrimp nauplii promoted the best combined growth and survival. Weight gains averaged 411 and 396%, and survival was 97 and 98% for experiments 1 and 2, respectively. Interestingly, the freeze-dried form of this diet (treatment 4a) produced much poorer survival, while growth was approximately one-eighth that achieved on the live form of this diet.

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The survival rates of groups of fish fed the same diet in both experiment 1 and 2 were very similar even though the size and age of the fish at the onset of each study was different. However, the diets produced better

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growth in experiment 2 fish than they did in experiment 1. Since both growth and survival were never as good as the live brine shrimp fed fish groups, it is very difficult to draw any direct correlation to the dietary effect on these parameters.

TABLE 13. PERCENT COMPOSITION OF THE FATTY ACIDS OF SILVERSIDES CULTURED FOR 37 DAYS ON VARIOUS DIETS (EXPERIMENT #2)

Fatty acid	24-day old	Experimental diets 2	3	4b	5	6	7	8	9	Wild Fish (72.90 mg)
14:0	1.44	1.21	1.32	1.69	1.67	3.08	1.00	2.67	3.20	1.34
14:1	0.55	0.74	0.22	0.40	0.06	-	0.24	0.12	0.12	0.14
15:0	0.74	0.81	0.60	0.42	0.36	0.60	0.13	0.50	0.54	0.88
15:1	-	-	0.13	0.03	0.02	0.14	0.06	0.10	0.18	0.14
16:0	19.45	15.59	23.50	22.09	17.57	19.76	15.37	19.67	21.21	22.55
16:1	7.70	14.70	trace	2.40	4.97	2.67	1.71	8.30	6.98	5.83
18:0	11.52	3.49	15.73	8.15	2.22	6.21	7.19	10.07	10.43	9.44
18:1ω9	32.23	35.83	30.50	21.84	27.44	25.75	31.96	26.31	28.15	10.40
18:2ω6	3.77	4.15	2.27	1.74	20.40	3.13	19.55	1.70	2.00	1.23
18:3ω6	0.17	0.51	0.14	0.12	3.86	0.41	0.83	0.14	0.05	0.38
18:3ω3	1.96	3.62	1.18	0.39	1.88	0.57	1.08	0.82	0.94	0.88
18:4ω3	0.58	0.57	0.18	0.29	0.66	1.47	0.87	0.45	0.59	0.75
20:0	-	-	0.70	-	-	-	1.49	-	0.13	-
20:1ω9	1.54	0.73	0.20	0.88	1.92	7.06	1.82	5.70	5.52	0.71
20:4ω6	5.12	3.83	5.43	3.58	1.75	0.80	1.20	2.00	1.86	3.75
20:5ω3	4.92	4.88	2.92	3.05	0.86	2.67	0.82	2.21	1.77	7.37
22:1	0.10	-	-	-	0.17	4.02	1.64	2.58	2.76	0.20
22:5ω3	2.11	2.74	3.53	4.12	1.65	4.54	0.88	0.75	1.55	1.34
22:6ω3	5.30	4.99	9.46	26.69	10.54	11.54	10.66	14.54	10.79	35.53
% oil	12.37	14.48	5.67	14.98	15.13	13.98	13.60	9.29	14.38	-
ω3/ω6 ratio	1.64	1.93	2.20	5.74	0.58	3.20	0.64	4.39	4.00	7.53
% survival*	-	98.00	32.00	65.00	80.00	58.00	46.00	77.00	72.00	-
weight gain*	-	206.6	-	107.8	80.40	34.10	49.60	4.60	14.60	-

* Data from Beck and Poston (in press). Weight increase in mg over 5.6 mg initial weight.

The whole body lipid level (19.9%) of the brine shrimp fed group was the highest of all the diet fed groups. From this data it appears that cultured animals were depositing large levels of lipids to accommodate the fatty acids needed in tissue development. Heck and Calbert (1977) found that laboratory cultured yellow perch (Perca flavescens) had more stored fat than wild fish and that fatty livers were common. Similar results were found in a comparative study of the anatomical and chemical characteristics of brine shrimp fed and wild herring, Culpea harengus (Balbontin et al., 1973). Cultured herring had total triglyceride levels which were 2.5% greater than wild fish levels. High triglycerides generally result from excess caloric intake, reduced

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activity, and/or poorly balanced diets, all factors which may explain the results found in this study.

Since the ω3 acids have been shown to be of chief importance in the metabolism of fish, it would seem that the amount of lipid storage may be related to a certain animal amount of ω3 PUFA. When the level of 22:6ω3 became low (1.95% in the 137-day-old fish, brine shrimp batch cultured study), the lipid storage increased drastically, indicating that a mechanism may exist which enhances the absorption and deposition of lipids to ensure minimal 22:6ω3 tissue levels. Therefore, it would seem likely that the concentration of 22:6ω3 may be the controlling factor for the deposition rate of ingested triglycerides. What affect an extended feeding of brine shrimp may have on the physiological and reproductive activities of the cultured silversides is not known.

FEEDING TRIAL 1977

Table 14 lists the diets fed in the two experiments for the 1977 feeding trials. These diets included the live, unfed, 3-day-old brine shrimp nauplii, the Cortland (Tunison) flake diet (Table 15) and the Meyers prawn diet #1023-77-1 (Table 16). The other treatments in the two experiments consisted of feeding regimes of artificial diets with brine shrimp nauplii at variable intervals.

TABLE 14. EXPERIMENTAL TREATMENTS AND FEEDING SCHEDULES (1977)

Treatment	Full Name	Abbreviated Name	Feeding Schedule
Experiment 1			
1	Meyers prawn diet (artificial-flake)	MPD	Every day
2	Live, unfed, 3-day-old brine shrimp	BrS	Every day
3	Starved	----	Unfed
4	Tunison modified trout diet (artificial-flake)	HPM-1F	Every day
5	Meyers diet + brine shrimp	MPD + BrS	MPD replaced with BrS every 2nd day
Experiment 2			
1	Meyers diet + brine shrimp	MPD + BrS	MPD replaced with BrS every 4th day
2	Live, unfed 3-day-old brine shrimp	BrS	Every day
3	Starved	----	Unfed
4	Tunison diet + brine shrimp	HPM-1F + BrS	HPM-1F replaced with BrS every 2nd day
5	Tunison diet + brine shrimp	HPM-1F + BrS	HPM-1F replaced with BrS every 4th day
6	Tunison diet + brine shrimp	HPM-1F + BrS	HPM-1F replaced with BrS every 8th day

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TABLE 15. COMPOSITION OF CORTLAND (TUNISON) DIET (HPM-1F)

Ingredient	Percent Composition
Herring meal	37.14
Soybean meal, 49% protein	9.28
Corn gluten meal, 60%	9.28
Wheat middlings, standard	8.36
Brewer's dried yeast	4.64
Dried whey	4.64
Dried condensed fish soluables	4.64
Meat and bone meal	4.64
Soybean oil, table grade*	4.64
Fish oil	4.64
Minerals mixture†	0.37
Vitamin mixture§	0.56
Choline chloride, 70%	0.002
Niacinamide	0.02
Starch, Instant Clear Jel, national	1.86
Lecithin	1.86
Cholesterol	0.19
Kelgin (HV) alginate	2.32
Sodium hexametaphosphate	0.93

* The soybean oil, fish oil, lecithin and cholesterol were blended and added to premix simultaneously.

† Mixture provided the following minerals (in gm/kg of diet): Magnesium sulfate, 1.78; Potassium iodate, 0.0076; Sodium selenite, 0.00027; Zinc sulfate, 0.4; Ferrous sulfate, 0.3; Cupric sulfate, 0.33; and Manganous sulfate, 0.889.

§ Mixture provided 13,800 IU Vitamin A palmitate; 5,500 IU Vitamin D_3; 100 IU Vitamin E, as dl-α-tocopheryl acetate, and the following amounts (mg) of other vitamins per kg of diet: Vitamin K as Menadione dimethylpyriminidol bisulfite, 30; thiamine·HCl, 28; d-biotin, 8; folic acid, 20; Vitamin B_{12}, 0.003; inositol, 691 and ethoxyquin, 207.

An additional diet, the Conklin modified lobster diet (CMLD) (Table 17) was included in only experiment #1 and was only evaluated from a fatty acid point of view (Schauer and Simpson, 1978). Therefore, it was not included in the general diet treatment (Table 14), although the performance of this diet will be included in the fatty acid results of this year's feeding trials. The CMLD diet had been proposed as a reference standard for nutritional studies of invertebrates.

Test fish in experiment #1 were 23 days old at the beginning of the experiment (17.6 ± 0.59 mg; 15.5 mm) and they were fed the various diets for 24 days. In experiment #2, fish were 59 days old (92.8 ± 0.85 mg; 25.8 mm) at the onset of the study and were fed the various diets for 50 days.

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TABLE 16. COMPOSITION OF THE MEYERS PRAWN DIET*†

Ingredient	Percent Composition
Shrimp meal (South)	15
Fish meal	14
Fish protein concentrate	15
Soy protein	15
Yeast protein	14
Rice gel	5
Whey	2
Linolenic acid	0.5
Fish oil (menhaden)	4
Cod liver oil	1
Soy oil	2
Cholesterol	0.1
Vitamin mix	2
Fish solubles	5
Lecithin	2
Kelgin	2.5
Sodium hexametaphosphate	1

* cf. I.B. Tarshis (1978).

† Proximate analysis: protein, 53.1%; fat, 11.7%; fiber, 4.7%; ash, 14.0%; carbohydrate, 16.5% (dry weight basis).

TABLE 17. COMPOSITION OF THE CONKLIN MODIFIED LOBSTER DIET

Ingredient	Percent Composition
Vitamin free casein	30.00
Cellulose	16.10
Wheat gluten	15.00
Brewer's yeast	15.00
Lipid mix S*	6.00
Corn starch	5.00
Albumin	4.00
Salt mix, BTM†	3.00
Vit. mix D§	2.00
Choline chloride	1.00
Thiamin	2.00
Cholesterol	1.00
Rovimix E	0.20
Vitamin C	0.20

* Corn oil, 2.03%; cod liver oil, 3.96%; and ethoxyquin, 0.01%.

† Salt mixture in % of mix: $CaCO_3$, 2.10%; $Ca(PO_4)_2$, 73.5%; citric acid, 0.21%; $2CU_2C_6 \cdot H_5O_7 \text{-} 5H_2O$, 0.05%; $FeC_6H5O_7 \text{-} 5H_2O$, 0.56%; MgO, 2.50%; KI, 0.001%; K_2HPO_4, 8.10%; K_2SO_4, 6.80%; NaCl, 3.06%, $Na_2HPO_4 \text{-} 2H_2O$, 2.14%; $7N_3(C_6H_5O_7)_2 \text{-} 2H_2O$, 0.13%; NaF, 0.002%; and $CoCL_2$, 0.02%.

§ Vitamin mixture in % of mix: thiamin hydrochloride, 0.32%; riboflavin, 0.72%; niacinamide, 2.56%; biotin, 0.008%; Ca-Pantothenate, 1.44%;

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TABLE 17. fn. cont.

Pyridoxine HCL, 0.24%; folic acid, 0.10%; mendione, 0.08%; Vitamin B_{12} (cobalamine, 3000 µg/g), i-inositol (meso), 12.5%; ascorbic acid, 6.00%; para-amino-benzoic acid, 2.00%; Vitamin D_2 (5000,000 usp/g), 0.04%; BHA and BHT, each 0.75%; Vitamin A (25,000 IU/g), 1.00%; celite, 72.58%.

The growth and survival data for silversides in experiments 1 and 2 are presented in Tables 18 and 19. In experiment 1, survival was best on the live brine shrimp diet, slightly less on the combination diet and poor on all artificial diets. Brine shrimp, as a single diet source, gave the most favorable growth of all treatments. Growth of brine shrimp (only) fed fish was significantly better than in the combination diet group, which in turn was significantly better than any of the artificial diets. The Conklin diet produced a survival rate of 78.7%, although growth was the poorest of all diet fed groups (22.8 mg ± 1.8; 17.3 mm).

TABLE 18. SURVIVAL AND GROWTH OF SILVERSIDES CULTURED (EXPERIMENT 1)*†

	Treatment groups (47-day-old fish)			
	1	2	4	5
% Survival	45.0	92.7	42.8	88.0
Final wet wt (mg) (X ± SE)	34.3 ± 1.5	104 ± 2.4	40.6 ± 2.0	87.7 ± 3.1
Final length (mm)	19.0	27.1	19.8	26.0

* See Table 14.
† Data after Beck and Bengtson (1979).

TABLE 19. SURVIVAL AND GROWTH OF SILVERSIDES CULTURED (EXPERIMENT 2)*†

	Treatment groups (109-day-old fish)				
	1	2	4	5	6
% Survival	89.3	90.7	78.3	90.7	86.7
Final wet wt (mg) (X ± SE)	372 ± 17.9	448 ± 21.1	426 ± 20.7	318 ± 16.5	302 ± 14.6
Final length (mm)	42.5	45.6	45.8	40.7	38.9

* See Table 14.
† Data after Beck and Bengtson (1979).

In experiment 2, survival was equally high in three of the diet groups. These were brine shrimp alone (treatment 2), Meyers diet + brine shrimp every 4th day (treatment 1), and Tunison diet + brine shrimp every 4th day (treatment 5). Survival was slightly poorer when fish were fed Tunison diet + brine shrimp every 2nd day (treatment 4) or 8th day (treatment 6). Statistically speaking, growth was best, and similar, on brine shrimp as a single diet source and Tunison diet + brine shrimp every 2nd day. Tunison diet + brine shrimp every 4th or 8th day were also statistically indistinguishable but exhibited the poorest growth (Beck and Bengtson, 1979). Growth on Meyers

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diet + brine shrimp every 4th day was intermediate to the previously mentioned, similar pairs. All other treatment pairs were statistically different.

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Amino Acids

The amino acid profiles of diets used in experiments 1 and 2 are presented in Table 20. The live, unfed, 3-day-old brine shrimp diet showed similar levels of most essential and non-essential amino acids as those of the artificial flake diets (MPD; HPM-1F).

Minimum whole body amino acid requirement levels of Chinook salmon (Oncorhynchus tshawytscha) (Mertz, 1969) were used as a standard reference to evaluate amino acid levels in the diets and cultured fish in this study.

Amino acid profiles for silversides cultured on one or more of the test diets for experiments 1 and 2 and for the wild fish are presented in Tables 21 and 22. In general, levels of essential and non-essential amino acids appear to be equally well maintained by silversides in all diet treatments, as evidenced by the likenesses of figures for any given amino acid.

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Fatty Acids

The major differences in the ω3 fatty acid composition of each diet made possible the evaluation of uptake and deposition of each diet's fatty acid component into the total lipids of the fish, even in combination diet feeding regimes. The brine shrimp contained 15.0% 18:3ω3 but no 22:6ω3 while the Tunison diet provided 1.5% 18:3ω3 and 8.7% 22:6ω3 (Table 20). The Meyers and Conklin diets had levels of fatty acids which were quite similar and generally the level of 18:3ω3 and 22:6ω3 was intermediate to the levels found in the brine shrimp and Tunison diets. All four diets contained nearly the same proportion of 20:5ω3. However, there was a substantial difference between the lipid levels: the Conklin diet contained 6.00% lipid (dry weight basis), the brine shrimp and Meyers diets contained approximately 9.00% lipid and the Tunison diet contained 12.00% lipid.

The major ω3 dietary fatty acids were incorporated into each fish culture group and deposited, seemingly unchanged (Tables 23 and 24). Therefore, each test group resembled its dietary fatty acid composition more than its original fatty acid composition. Similarly, the fatty acids in the combination diet fed fish nearly reflected the average of the major fatty acid differences in the brine shrimp and the artificial diets. This indicates that the artificial diet was being assimilated and deposited in a similar manner as the live diet. All of the artificial and combination diet fed groups contained higher levels of 22:6ω3 than groups fed brine shrimp as a single diet source. Nevertheless, the level of 22:6ω3 was never more than one-third the level of the wild fish of a similar size. Also, the wild fish fatty acid profile showed a substantially lower level of 16:1ω7 and 18:1ω9 and the total lipid levels of the cultured fish were nearly twice those of the wild fish.

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TABLE 20. AMINO ACID AND FATTY ACID COMPOSITION OF THE EXPERIMENTAL DIET

Amino Acid*	Chinook Salmon Req.†	BrS	MPD	HPM-1F	Fatty Acid Methyl Esters§	BrS	MPD	HPM-1F
Aspartic acid	---	8.0	11.1	11.0	14:0	0.81	5.81	3.25
Threonine	2.5	4.5	4.4	5.0	16:0	11.46	18.43	13.26
Serine	---	4.6	4.9	4.9	16:1ω7	9.11	8.98	5.82
Glutamic acid	---	11.1	17.5	17.4	16:3ω4/17:1ω8	1.00	1.48	1.52
Proline	---	5.6	7.8	7.9	18:0	4.42	3.07	4.21
Glycine	---	4.9	6.1	6.5	18:1ω9	37.50	16.47	26.15
Alanine	---	5.7	6.7	7.7	18:2ω6	5.84	21.36	18.00
½ Cystine	---	1.0	---	---	18:3ω3	15.00	5.35	1.51
Valine	3.25	4.8	3.0	2.8	18:4ω3	2.99	1.33	1.25
Methionine	1.5	2.2	2.3	3.1	20:1ω9	0.83	1.76	2.78
Isoleucine	2.5	4.5	4.9	5.1	20:3ω3/20:4ω6	1.65	---	---
Leucine	4.0	7.1	8.5	10.0	20:5ω3	7.22	6.92	7.88
Tyrosine	---	7.3	3.2	3.4	22:1	---	---	3.21
Phenylalanine	5.25	5.3	4.8	4.9	22:5ω3	---	0.78	0.17
Histidine	1.75	3.4	2.5	2.4	22:6ω3	---	3.98	8.68
Lysine	---	7.5	7.4	8.1				
Tryptophan	0.5	---	---	---				
Arginine	5.75	6.1	6.3	6.1	% lipid††	9.40	8.90	12.10

* Expressed as g amino acid per 100 g protein.

† Minimum whole body amino acid requirement levels of Chinook salmon (E.T. Mertz, see References).

§ Weight percent values.

††Based on dry weights.

Data in Tables 20, 21, 22, 24 from Seidel, C.R., P.S. Schauer, T. Katayama and K.L. Simpson. 1980. Bull, Jap. Soc. Sci. Fish. 46:237-245.

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TABLE 21. AMINO ACID PROFILES FOR SILVERSIDES (EXPERIMENT 1)
(Amounts expressed as g amino acid per 100 g protein)
(Tryptophan destroyed in HCl hydrolysis)

Amino Acid	24-day-old silversides	Treatment Groups 1	2	3	4	5	Wild silversides*
Aspartic acid	9.7	8.9	9.9	9.0	9.1	8.7	9.3
Threonine	4.4	4.4	4.4	4.1	4.5	4.3	4.2
Serine	4.4	4.1	4.1	4.1	4.2	4.3	4.0
Glutamic acid	14.3	14.4	13.8	13.5	14.3	13.4	14.6
Proline	4.2	4.6	9.1	8.1	4.5	4.8	7.0
Glycine	6.0	7.4	6.8	6.9	7.0	6.4	8.2
Alanine	6.5	6.1	7.0	6.2	6.4	6.4	7.0
½ Cystine	---	0.1	0.4	0.3	0.2	0.6	0.7
Valine	5.3	7.0	5.8	4.8	5.8	4.9	6.6
Methionine	2.9	2.3	2.6	3.2	3.1	2.9	2.9
Isoleucine	4.1	4.2	4.4	3.9	4.0	4.2	4.0
Leucine	7.6	7.7	7.9	7.4	7.3	7.4	7.7
Tyrosine	3.4	3.6	3.6	3.5	3.6	3.4	3.3
Phenylalanine	4.0	4.2	4.8	4.0	4.1	4.1	4.1
Histidine	4.2	3.5	2.9	3.1	3.0	3.0	3.4
Lysine	9.1	7.9	7.5	9.5	8.0	9.3	9.2
Arginine	6.8	7.0	7.0	6.3	6.3	6.2	7.0

*Wild silversides were 62.8 mm; 1.36 g.

TABLE 22. AMINO ACID PROFILES FOR SILVERSIDES (EXPERIMENT 2)
(Amounts expressed as g amino acid per 100 g protein)
(Tryptophan destroyed in HCl hydrolysis)

Amino acid	59-day-old silversides	Treatment Groups 1	2	4	5	6	Wild silversides*
Aspartic acid	9.4	10.3	11.9	11.0	10.4	10.1	9.3
Threonine	4.3	4.4	4.8	5.0	5.0	4.9	4.2
Serine	4.3	3.8	4.1	4.5	4.8	5.2	4.0
Glutamic acid	13.9	15.6	15.8	16.0	15.9	17.9	14.6
Proline	4.8	5.7	4.6	7.0	6.1	4.0	7.0
Glycine	6.5	8.1	6.7	7.2	7.4	7.8	8.2
Alanine	6.4	6.8	4.2	7.0	7.0	7.8	7.0
½ Cystine	0.5	0.3	---	0.4	0.7	---	0.7
Valine	5.3	5.5	5.2	5.2	5.8	8.2	6.6
Methionine	2.7	3.3	3.2	2.9	3.3	3.1	2.9
Isoleucine	4.1	4.4	4.8	5.7	5.2	4.3	4.0
Leucine	7.3	8.8	8.7	9.0	9.3	8.2	7.7
Tyrosine	3.4	3.3	3.1	3.4	3.4	3.6	3.3
Phenylalanine	4.2	4.8	3.8	4.4	4.6	5.8	4.1
Histidine	3.2	3.5	4.6	2.8	3.1	2.9	3.4
Lysine	8.0	7.9	9.0	8.7	9.4	8.7	9.2
Arginine	6.5	7.3	6.4	7.4	7.0	7.7	7.0

*Wild silversides were 62.8 mm; 1.36 g.

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TABLE 23. MAJOR FATTY ACIDS FROM TOTAL LIPIDS OF CULTURED AND WILD SILVERSIDES (EXPERIMENT 1)*

Fatty acid†§ Methyl esters	24-day-old silversides	Treatment Groups 1	2	3	4	5	Wild †† silversides
14:0	0.80	1.82	1.44	0.59	1.56	2.23	1.50
16:0	12.63	20.79	11.93	15.35	19.94	14.51	26.52
16:1ω7	12.10	5.99	9.79	6.14	4.87	8.36	7.76
16:2ω4/ω7	0.64	0.49	0.74	0.71	1.14	0.80	3.18
16:3ω3/ω4	1.69	----	1.52	1.15	0.72	1.35	----
18:0	4.68	10.03	5.40	8.18	4.75	4.91	9.25
18:1ω9	35.81	20.67	29.81	26.79	26.32	24.56	13.98
18:2ω6	8.62	12.27	7.05	5.89	13.69	13.31	0.50
18:3ω6	----	0.01	0.37	----	----	1.20	----
18:3ω3	10.26	1.69	10.24	4.17	1.09	6.22	0.48
18:4ω3	2.35	0.04	2.46	0.49	0.32	1.76	----
20:1ω9	0.34	1.40	0.76	0.70	2.79	1.01	1.07
20:3ω3/20:4ω6	2.31	2.29	3.40	6.99	2.02	2.63	3.25
20:5ω3	4.00	1.48	4.53	4.55	2.54	3.39	4.67
22:5ω3	0.60	1.00	2.13	3.52	0.33	1.07	----
22:6ω3	2.96	19.12	5.96	14.31	17.47	11.31	28.07
% body lipid§§	11.80	14.44	15.54	9.83	17.90	16.50	12.40

* Data from Schauer and Simpson, 1978.

† Weight percent values.

§ Fatty acids of less than 1.0% are: 14:1, 15:0, 15:1, 20:2ω6/ω9 and 22:1.

††Wild silversides were 25.7 mm; 87.6 mg.

§§Based on dry weight values.

TABLE 24. MAJOR FATTY ACIDS FROM TOTAL LIPIDS OF CULTURED AND WILD SILVERSIDES (EXPERIMENT 2)

Fatty acid*† Methyl esters	59-day-old silversides	Treatment Groups 1	2	4	5	6	Wild § silversides
14:0	1.17	3.57	1.25	2.33	2.04	2.44	1.06
16:0	12.07	22.42	16.25	19.52	22.01	22.59	20.49
16:1ω7	10.01	13.30	13.38	11.58	9.98	9.41	6.34
16:3ω4/17:1ω8	1.30	0.80	1.73	1.39	1.04	0.94	----
18:0	5.75	4.60	4.92	5.22	4.20	5.31	7.61
18:1ω9	30.47	31.81	38.20	37.50	35.30	35.10	15.67
18:2ω6	5.74	8.53	4.68	5.08	6.23	7.61	3.52
18:3ω3	10.12	1.56	5.58	1.69	0.87	0.74	0.72
18:4ω3	3.01	1.10	1.48	1.29	1.11	1.10	0.22
20:0	----	1.59	0.89	1.98	1.91	1.87	0.04
20:1ω9	0.90	1.29	0.53	1.63	1.83	2.19	1.57
20:3ω3/20:4ω6	2.88	0.80	1.88	1.13	1.12	0.36	5.41
20:5ω3	4.47	1.54	3.04	2.23	1.96	1.46	5.24
22:5ω3	2.70	0.21	1.11	0.35	0.41	0.39	2.58
22:6ω3	6.67	5.20	3.28	4.67	7.92	5.94	24.38
% body lipid††	17.32	15.83	15.12	15.28	14.30	16.30	7.99

* Weight percent values.

† Fatty acids of less than 1.0% are: 14:1, 15:0, 15:1, 16:2ω4 or ω7 and 22:1.

§ Wild silversides were 62.8 mm; 1.36 g.

††Based on dry weight values.

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SECTION IV

DISCUSSION

The overall approach to this project was to formulate an artificial diet for Atlantic silversides such that this fish could be cultured as a representative test organism. Toward this end, a comprehensive project was undertaken with the close cooperation of the Tunison, URI and EPA laboratories. Within the time frame it was not possible to critically determine the minimum levels of various nutrients. It was hoped that acceptable levels could be determined which would allow a diet to be constructed for this marine fish which would give good survival and growth and give a biochemical composition similar to that of the wild fish.

Initially, data were thus gathered on the composition of the silverside egg and wild fish, plankton, brine shrimp and various commercial and modified diets. While some improvements were made in the artificial diets, generally these diets did not produce the good growth and survival which resulted with a brine shrimp diet. Freeze-dried silversides and brine shrimp were included in the poor diet groups. The exact reason for these differences is not known, however we believe that certain factor(s) may be eliminated.

In the above-mentioned diet studies and in the combination live and artificial diet studies, silversides actively consumed all diets which seems to eliminate leaching as a major cause of nutritional incompleteness of artificial diets or freeze dried brine shrimp. It follows that if a critical nutrient were leached out, feeding brine shrimp every 8th day would give results similar to feeding an artificial diet alone, which was not the case. The reasonable growth and survival which resulted from feeding brine shrimp every 8th day also tends to eliminate a digestive enzyme as the key growth factor since an enzyme would not likely remain active over an 8-day period. Freeze drying would not substantially destroy digestive enzymes, amino acids or fatty acid components of the brine shrimp, yet feeding freeze-dried brine shrimp gave results equally as poor as feeding an artificial diet alone. It appears unlikely from our present knowledge, therefore, that the live brine shrimp is providing a digestive enzyme, amino acid or fatty acid to silversides that is not being provided by the artificial diets or freeze-dried brine shrimp.

We now know that the various test diets provided the majority of the 10 essential amino acids in quantities at, or above those noted as adequate. The phenylalanine requirement which was apparently not met by some artificial diets was probably spared by the tyrosine level (Halver, 1973) which was adequate. The artificial diets also appeared slightly low in valine, however the differences did not appear to negatively influence the cultured

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silversides. The whole body amino acid profiles for every essential amino acid of all cultured fish were in excess of the minimum requirement levels (Tables 21 and 22).

It has been observed that protein, unlike lipid deposition, is independent of dietary intake, and further that changes in dietary energy level or in relative dietary levels of protein, carbohydrate and fat do not affect the body protein (Lee and Putnam, 1973; Adron et al., 1976). This can be attributed to the fact that fish and other vertebrates are unable to store significant quantities of protein and carbohydrate (Buckley and Groves, 1978). The experimental results presented here seem to support the above observations since there were, with very few exceptions, similar amino acid levels found in all cultured silversides, regardless of their diet. In fact, the essential amino acids in the test diets were in excess of their requirement level and were probably not incorporated into fish flesh. Therefore, the differences observed in growth and survival of silversides cannot be attributed directly to amino acid deficiencies of the diet.

It was shown that a combination of the Meyers + brine shrimp diet produced a fatty acid spectrum in silversides which was nearly identical to the average spectra of each single source diet (Meyers alone; brine shrimp alone) (Table 23). The resemblance of fish whole body fatty acids to their dietary fatty acid composition has been well illustrated by Lovern (1950), Lee et al. (1967), Owen et al. (1972), and more recently (in Atlantic silversides) by Schauer (1977).

Two important differences were evident between the cultured and wild fish lipids. The first was that the cultured fish contained a greater amount of the 16 and 18 carbon fatty acids, whereas the wild fish contained more of the 20-22 carbon polyunsaturated fatty acids (PUFA). It appeared that silversides accumulated dietary fatty acids, without biosynthetic elongation and desaturation, when there were sufficient levels of the long chain PUFA in their diet to maintain tissue integrity and development. An induced biosynthetic activity in treatment #2 (1977) fish, which was perhaps caused by low levels of the long chain PUFA in the brine shrimp diet, may be the reason for the lack of accumulation of 18:3ω3 and 20:5ω3. However, further study is required to evaluate this relationship.

Linolenic acid (18:3ω3) was found to be the essential fatty acid of rainbow trout (Castell et al., 1972a,b; Watanabe et al., 1974a,b,c). However, the importance of the longer chain PUFA (20:5ω3 and 22:6ω3) in marine fish diets has been shown by Yone and Fujii (1975) in red sea bream (Chrysophys major), Takeuchi and Watanabe (1976, 1977a, 1978) in rainbow trout (Salmo gairdneri), and Takeuchi and Watanabe (1977b) in carp (Cyprinus carpio L.). Our data implies that these long chain PUFA may be of at least equal importance for good growth of marine fish due to their predominance in tissue lipids of the wild fish and their selective retention in the starved fish (Table 23).

The second major difference between the cultured and wild fish was that in the former group total lipid levels were substantially higher than in the latter group. A similar occurrence was reported by Balbontin et al. (1973)

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in cultured herring (Clupea harengus). A direct correlation between dietary lipid levels and total body lipid has been shown in channel catfish (Page and Andrews, 1973), and in plaice (Cowey et al., 1975). Furthermore, Poston (1975) showed a linear increase in body fat of rainbow trout as dietary energy levels increased.

Live brine shrimp have provided some added requirement for maintaining good survival. With this increased survival, the nutritional requirements of marine larval fish can now be more accurately evaluated by feeding modified artificial diets. It would appear that brine shrimp cannot be totally eliminated from the dietary regime of the larval fish, but they can be more conservatively utilized.

It is thus possible to supplement the fatty acid content of the brine shrimp so that the resulting fish does survive, grow and more closely resemble the natural (wild) fish than do fish grown on only the live brine shrimp nauplii. This can be done without having to maintain the fish on a total live diet.

We have stated above that the leaching of nutrients from the artificial diets did not appear to be the major cause of these diets' poor performance. Nevertheless, one would expect losses of water-soluble constituents on contact with water. Goldblatt et al. (1979 - op. cit. Beck, Vol 11, p. 117) reported the leaching losses of several diets including dried brine shrimp and the "Conklin" diet used in this study. It was shown that weight losses in the diets were essentially constant after 20 min. Most non-capsulated diets showed large losses of most nutrients after one hour. As these authors point out, "If leaching occurs, and is unaccounted for, the consumed ration is no longer the formulated ration, and hence the conclusions are at best tentative."

The water content of the various diets in this study could be considered as a potentially significant variable. Bromley (Aquaculture 20, p. 91, 1980) studied the effect of the dietary water content on the growth and food conversion efficiency of the juvenile turbot. This author concluded that the formulation of the basic nutrients was important but that the water content (0-74%) was immaterial to growth and feed conversion. The effect of the water content of the diets was not studied in the present study. It would appear that the juvenile turbot (9-14 cm) does not have a requirement for a moist diet. It remains to be seen if these data can be extrapolated to larval fish of a different species.

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SECTION V

CONCLUSION

The intent of this project, started in 1975, was to provide an artificial diet that would give good growth and survival of test fish. These fish were to resemble the environmental fish. Generally speaking, all of the artificial diets--commercial or prepared, flaked or pelleted, and freeze-dried silversides or brine shrimp--gave poor survival and/or growth. Only live brine shrimp nauplii, or a combination of brine shrimp nauplii, gave acceptable survival and growth. Nevertheless, brine shrimp feedings resulted in a test organism that did not resemble its wild counterpart. However, a combination feeding of an artificial diet and brine shrimp gave good survival and growth and appeared to correct some of the deficiencies of brine shrimp alone. While the silversides were not completely "weaned" from the expensive (time and cost) brine shrimp, at least it was minimized.

It was never envisioned when this project began that the authors and the EPA group would embark on a study of brine shrimp cysts. At a conference in Szymbark, Poland, where some of these data were presented, an International Study of Artemia (ISA) group was formed. This group was formed in response to the obvious need to study the properties of brine shrimp, in general, and some commercial strains, in particular. The same close cooperative effort between the EPA and URI laboratories in the first project continued into the second. The comparative studies on the five geographical strains of brine shrimp were presented at the 1st International Symposium on Artemia (1979, Corpus Christi, TX) in seven papers from the two laboratories. The results showed clearly that there is wide variation in the brine shrimps' ability to support the life and growth of test organisms and also a variation in their chemical composition. The cause of the variability of biological value of brine shrimp is the subject of further investigation between the two laboratories.

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